

Docket No.: : 55046 (70207)
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: C.T. Walsh

Application No.: 10/017,324

Confirmation No.: 8192

Filed: December 15, 2001

Art Unit: 1656

For: METHODS FOR PREPARATION OF
MACROCYCLIC MOLECULES AND
MACROCYCLIC MOLECULES PREPARED
THEREBY

Examiner: Nashed, Nashaat T.

Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR 1.132

The undersigned declares as follows:

1. I, Christopher T. Walsh, Ph.D., declare that I am a resident of the United States of America. My residential address is 735 Washington Street, Wellesley, MA 02482.

2. I am an inventor on the above-identified application (hereafter, "the Application"), assigned to the President and Fellows of Harvard College. I am currently the Hamilton Kuhn Professor of Biological Chemistry and Molecular Pharmacology at Harvard Medical School. I have over 35 years experience in the area of biological catalysis, focusing on biosynthesis and mechanisms of action. I have published 600 papers in the area of biological catalysis, focusing on biosynthesis and mechanisms of action, and am an inventor on 3 patent applications. My curriculum vitae is attached as Exhibit 1.

3. It is my understanding that the Examiner has rejected claims 1-53, 55, and 59 of the Application under 35 U.S.C. § 112, first paragraph, as allegedly lacking written description for a structure activity relationship between the substrate to be cyclized and the thioesterase or thioesterase domain to be used for the cyclization reaction. The Examiner contends that: (a) one of ordinary skill in the art would not know which other thioesterase domain can function on its own as an excised fragment (the specification provides one example); and (b) the rationale in the rational design is not taught in the specification.

4. We, the inventors and Applicants, have discovered that an excised Type 1 thioesterase or thioesterase domain can function on its own as an excised fragment to prepare macrocyclic molecules, thereby obviating traditional synthetic chemistry approaches to macrocyclic molecule synthesis, which generally exhibit low yields, require protecting groups and typically are carried out in organic solvents.

5. As discussed in our application, many thioesterases are known. An enormous range of medicinally important polyketide and peptide natural products assembled by modular polyketide synthases (PKSs), non-ribosomal peptide synthetases (NRPS) and mixed PKS/NRPS systems have macrocyclic structures, including the antibiotics erythromycin (PKS) and daptomycin (NRPS), the immunosuppressants cyclosporin (NRPS) and rapamycin (PKS/NRPS) and the antitumor agent epothilone (PKS/NRPS). PKSs and NRPSs are very large multifunctional proteins that are organized into sets of functional domains termed modules (Cane et al, *Science* (1998) 282:62-8; Marahiel et al, *Chem. Rev.* (1997) 97:2651-74). The 6-deoxyerythronolide B synthase (DEBS) protein is a multidomain PKS protein with an integral TE domain that catalyzes cyclization of a protein-bound polyketide. Modification of domain identity or sequence in the natural DEBS protein by single or multiple domain substitutions or insertions of natural heterologous subunits generates DEBS protein variants that produce compounds with various ketide unit sequences.

6. The present invention teaches a method of preparing macrocyclic molecules from linear precursors. More specifically, the present invention features a method for the cyclization of linear substrates wherein macrocyclic ring-closure is effected preferably by the formation of an amide or an ester bond catalyzed by a thioesterase domain excised and expressed from the DNA sequence for non-ribosomal peptide synthetase (NRPS) or polyketide synthase (PKS) multidomain proteins.

7. We discovered that the excised thioesterase (TE) domain protein catalyzed cyclization reaction of an acyclic substrate having both an activated acyl group and a nucleophile, wherein the cyclization formed a macrocyclic product. Once that is taught, those skilled in the art will be able to practice the invention for any excised thioesterase (TE) domain protein.

8. The alleged selectivity of the thioesterase domain protein is provided by the inclusion of an end group functionality of the natural substrate for the TE domain. Examples of such TE domain proteins are clearly described at page 11 of the specification. The specification provides numerous examples of substrates that are amenable to the cyclization reaction mediated by a TE domain protein. Such substrates, found at least at pages 6-9 of the specification, are described by various generic formulae having common functional group characteristics.

9. The specification provides for a TE domain protein catalyzed cyclization of a substrate, wherein the substrate has a functional group preference for the TE domain protein, and a substrate structure that is clearly described by the specification.

10. TE domain proteins will catalyze cyclization of a specific substrate. Our invention enables those skilled in the art to use any TE domain protein, which is known to cyclize a specific substrate, for preparation of an appropriate macrocyclic molecule that

corresponds to the natural substrate.

11. We teach that any TE domain proteins will catalyze cyclization of a specific substrate are useful in the practice of our invention. All that is necessary for the skilled person is to have a TE domain proteins will catalyze cyclization of a specific substrate. The specification teaches to purify excised protein and contact with a substrate molecule for cyclization.

12. To determine a substrate for a particular TE domain protein one need only look at the natural substrate. Such a determination, if the natural substrate is not known, would be routine experimentation.

13. The Examiner states that the thioesterase domain is expected to display substrate selectivity. That is true, and it is exactly that substrate selectivity that is used in the present invention. Again, the discovery here was that the excised thioesterase domain protein can be used to catalyze cyclization of macromolecules.

14. It is my understanding that the Examiner has rejected claims 34-53 and 55 of the Application under 35 U.S.C. § 112, first paragraph, as allegedly lacking written description for a selection of an enzyme and substrate in the claimed cyclization reaction, wherein the substrate is initially elongated. We have provided a working example of such an elongation-cyclization reaction to produce gramicidin S., which has been acknowledged by the Examiner. However, it is further alleged that the specification does not provide teaching on the selection of enzymes and substrate or provide several other working examples showing the method is general in nature.

15. As explained above, the present invention is not in selection of enzymes and substrates. The present invention uses know thioesterases with their known substrates. The invention teaches that the thioesterase can be used to cyclize molecules having an activated acyl residue and a pendant nucleophile that corresponds to the natural substrate, instead of a chemical

reaction for the cyclization.

16. That is exactly what is described in the specification.

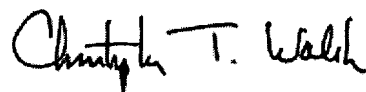
17. It is my opinion based on my knowledge and experience in the art that one skilled in the art can practice the present invention with any purified excised thioesterase (TE) domain protein with a substrate for that thioesterase (TE) domain protein having an activated acyl residue and a pendant nucleophile separated by a linear backbone.

18. Elongation of an organic substrate involves reaction sequences known to those of ordinary skill in the art, and is a matter of functional group conversions and couplings of at least two reagents. Although the elongation/cyclization process is in competition with hydrolysis as noted by the Examiner, the elongated/cyclized macromolecules can be readily separated from hydrolyzed molecules.

19. Based on my knowledge and experience in the art, it is my opinion that the present specification clearly enables one skilled in the art to practice the invention for the scope of the claims by disclosing multiple examples of TE domains appropriate to catalyze the macrocyclization reactions for substrates corresponding to the natural substrates of the enzymes. See, Trauger, et al. reference (Trauger, *Nature* (2000) 407: 215-218), which is cited on page 11 of the specification. Trauger, et al. state that PKS systems can produce new polyketides and peptides. See page 216, 1st column of Trauger et al. In addition, as the specification states at the top of page 8, substrate specificity of other excised TE domains [if not known] can be determined by those skilled in the art by routine procedures. It is my understanding that routine procedures are not considered “undue experimentation”. At most, only routine procedures are needed to determine substrates.

20. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both (18 U.S.C. 1001), and that such willful false statements may jeopardize the validity of the above-identified Application or any patent issued thereon.

Date: April 11, 2007



Christopher T. Walsh



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Pharmacology Department,
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EDUCATION

B.A. Harvard College, 1965 Biology

Ph.D. The Rockefeller University 1970 Life Sciences

Helen Hay Whitney postdoctoral fellow, 1970-1972 Biochemistry Department
Brandeis University (Prof. R.H. Abeles)

PROFESSIONAL EXPERIENCE

1972-1987	Assistant, Associate and Full Professor of Chemistry and Biology, MIT
1980-1985	Uncas and Helen Whitaker Professor, MIT
1982-1987	Chairman, MIT Chemistry Department
1985-1987	Karl Taylor Compton Professor, MIT
1987-1995	Chairman, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School
1992-1995	President, Dana-Farber Cancer Institute
1991-	Hamilton Kuhn Professor of Biological Chemistry and Molecular Pharmacology, Harvard Medical School

PROFESSIONAL ACTIVITIES

1977-1979	Panel Member: NSF Research Grants Study
1978-1980	Editorial Board: <i>Journal of Biological Chemistry</i>
1978-1980	Biology Section Editor: <i>Annual Reports in Medicinal Chemistry</i>
1978-1982	Panel Member: NIH Study Section in Biochemistry; Chair in 1982
1978, 1984	Co-chairman: 1978 Gordon Research Conference on Enzymes, Coenzymes, and Metabolic Pathways; 1984 Conference on Methanogenesis
1991-1992	Associate Editor: <i>Protein Science</i>
1990-1995	Associate Editor: <i>Annual Review of Biochemistry</i>



1991-1994	Editorial Board: <i>Journal of the American Chemical Society</i>
1993-	Editorial Board: <i>Chemistry and Biology</i>
1996- 1999	Member, NIH General Medical Sciences Advisory Council
2001- 2006	<i>Science</i> , Board of Reviewing Editors
2003-	Editorial Board, <i>Organic Letters</i>
2005-	Editorial Board, <i>ACS Chemical Biology</i>

SOCIETY MEMBERSHIPS AND AWARDS

Alfred P. Sloan Foundation Fellow, 1975-1977
Camille & Henry Dreyfus Teacher-Scholar Grant Recipient, 1976-1980
Eli Lilly Award in Biochemistry, 1979
American Academy of Arts and Sciences, elected 1988
Institute of Medicine (elected 1989)
National Academy of Sciences (elected 1989)
Centenary Medal & Lectureship, Royal Society of Chemistry
Arthur C. Cope Scholar Award, Am. Chem. Soc., 1998
Repligen Award (for Chemistry of Life Processes) Am. Chem. Soc , 1999
Bader Award (for Bioorganic Chemistry), Am Chem Soc, 2003
American Philosophical Society, elected 2003
Promega Award, American Society of Microbiology, 2004
Fritz Lipmann Award, Am Soc Biochem & Mol Biol, 2005
Murray Goodman Award, Biopolymers/Am Chem Soc 2007

ORGANIZATIONS

1985-1988	Member, Visiting Committee in Biological Sciences, Yale University
1984-1986	Member, Visiting Committee in Chemistry, Princeton University
1988-1992	Scientific Advisory Committee, Children's Hospital; Chairman
1991-1992	
1989	Scientific Advisory Board, Burroughs Welcome Fund in Experimental Therapeutics
1991-1994	Cardiovascular Research Center Advisory Committee, Massachusetts General Hospital
1993-1996	Board of Directors, Association of American Cancer Institutes
2000-	Structural Biology Review Group, HHMI
1998-2004	Board of Directors, Whitehead Institute
2001-	Board of Directors, Helen Hay Whitney Foundation

PHARMACEUTICAL CONSULTING

1975-1981	Merck
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1981-1996	Roche
1981-1997	Abbott
2007-	Eisai

BIOTECHNOLOGY COMPANIES

1981-2001	Scientific Advisory Boards during this interval: Immunogen, Genzyme, Cambridge Neurosciences, Epix Medical, Millennium Pharmaceuticals, Dyax, Health Care Ventures, Caliper
2000-2005	Medical and Scientific Advisory Board, MPM Capital
2006-	Scientific Advisory Board Health Care Ventures
2004-	Scientific Advisory Board ; Sirtris

Board of Directors:

- Leukosite (1996-2001)
- Diacrin (1998-2000)
- Kosan Biosciences (1996-)
- Versicor/Vicuron (1997-2005)
- Transform Pharmaceuticals (2000-2005)
- Critical Therapeutics (2001-)
- Microbia (2003-)
- Magen Biosciences 2006-

PUBLICATIONS

665 research publications in enzymology, natural product biosynthesis

Books: C. Walsh *Enzymatic Reaction Mechanisms* (1979) W.H. Freeman Press (San Francisco)

C. Walsh *Antibiotics: Origins, Actions, Resistance* (2003) ASM Press (Washington)

C. Walsh *Posttranslational Modification of Proteins: Expanding Nature's Inventory* (2005) Roberts & Co. (Colorado)

COWORKERS

67 PhD students supervised

130 postdoctoral fellows supervised